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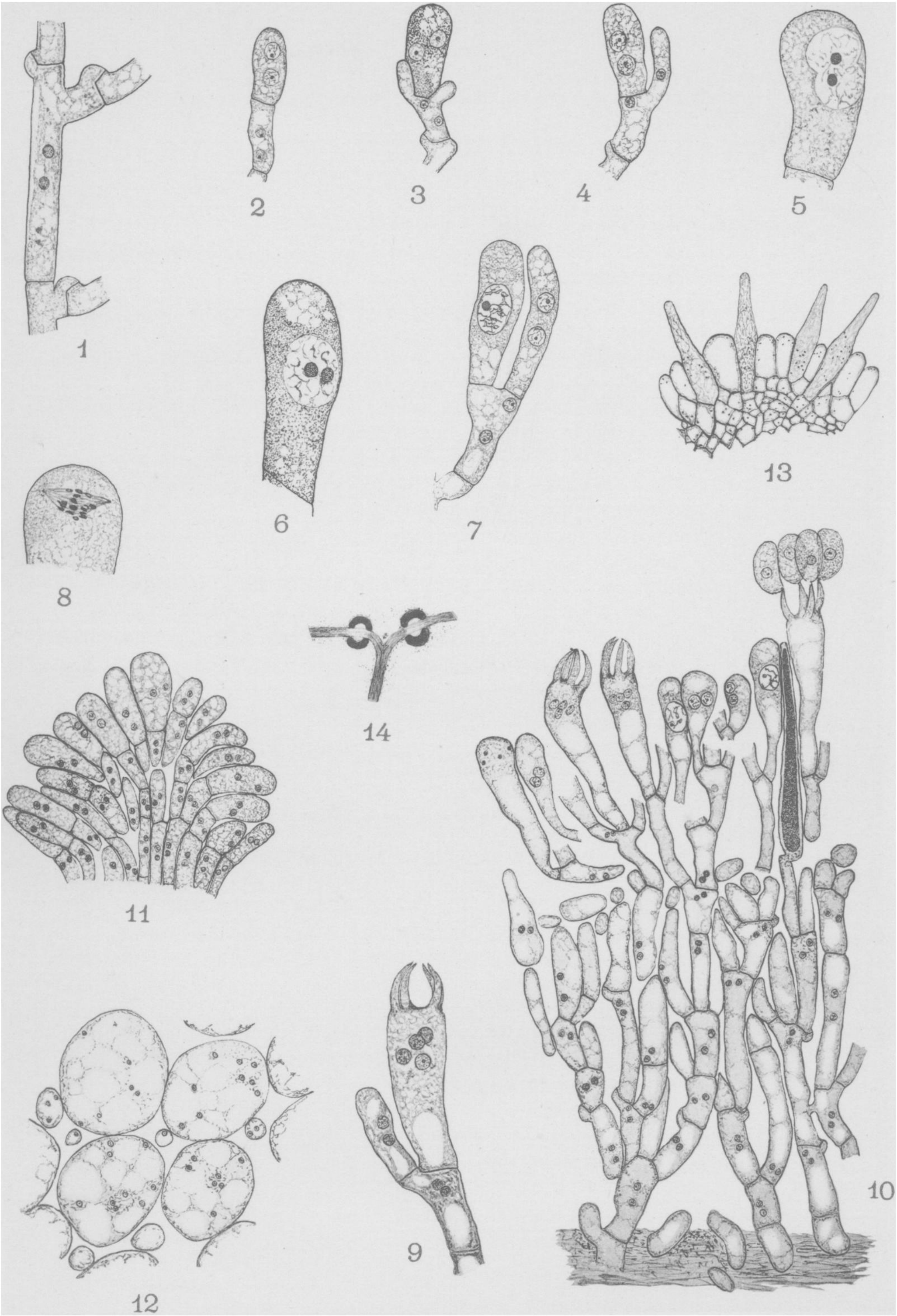

HARPER on BINUCLEATE CELLS.

VOLUME XXXIII NUMBER 1

BOTANICAL GAZETTE

JANUARY, 1902

BINUCLEATE CELLS IN CERTAIN HYMENOMYCETES.

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(WITH PLATE I)

INCLUDING the basidia and spores, Rosenvinge found that the cells of the Basidiomycetes contain from one to many nuclei. He was of the opinion that the uninucleated condition is typical and that only the uninucleated cells divide, the multinucleate condition arising secondarily by division of the originally single nucleus of each cell.

Two notes by R. Maire (**9**, **10**) in the *Comptes Rendus* of July 9 and December 24, 1900, report that the hyphal cells of the young carpophore in a large series of Hymenomycetes are regularly binucleated. This discovery throws a new light on the question as to the nature of the nuclear fusions in the basidium, and indeed on the whole question of the morphology of the carpophore. In view of the many times suspected relationship of the Basidiomycetes and Ascomycetes and the seemingly natural assumption of the equivalence of the nuclear fusions in the ascus and basidium, my own work on the ascus led me to desire further confirmation of the results given by Maire, and especially further light on the number and nature of the nuclei in the mycelium as well as the carpophore of the Hymenomycetes. In his first note Maire describes observations on a series of nine genera of Agaricineae, Polyporeae, etc. In all these genera he finds that the mycelial cells of the young carpophore are binucleated. In the mature carpophore the majority of the cells in the stipe and pileus are multinucleate, but this condition arises from the amitotic fragmentation of the two nuclei originally present in each cell. The subhymenial cells from which the basidia spring and the paraphyses are always binucleate. This involves the remarkable fact that all the cells which are directly concerned in producing the basidiospores are throughout their development binucleated. In other words, all the cells of the *Keimbahn* are binucleated, the multinucleated condition only arising in cells of strictly limited development and found in the organs of nutrition, support, transportation, etc. Maire also finds that the pairs of nuclei divide simultaneously as "conjugate nuclei" (12), so that in the successive cell generations which arise in the development of the carpophore the two nuclei in each cell are always of widely separated nuclear ancestry, the whole condition duplicating exactly what has been described by Dangeard and Sappin-Trouffy (3) and Poirault and Raciborski (12) for the rusts. Further, in the young basidium two and only two nuclei are always present, just as in the teleutospore of the rust. These two nuclei fuse to form the primary nucleus of the basidium, which then divides twice to furnish the nuclei for the typically four basidiospores. The descriptions of Rosen (13) and Wager (16, 17), according to which a series of nuclei fuse successively in pairs to form the primary nucleus of the basidium, are entirely incorrect according to Maire for the large series of forms examined by him.

In his second note Maire describes observations on a series of Gasteromycetes, in which he finds exactly the same conditions as described above for the Hymenomycetes.

Maire believes that the fusion of nuclei in the basidium is without doubt sexual in its nature, since the pronuclei which unite are of widely separated origin, although passing the whole course of their development in a single cell series. These facts bring the carpophore of the Basidiomycetes into closest parallelism with the whole life cycle of the Uredineae, both as to the nature of their vegetative cells and in the similarity of the processes in the basidium with those in the teleutospore and promycelium. The only marked difference is in the fact that the promycelium of the rusts is a septate basidium with lateral spores instead of a one-celled basidium with apical spores. Should Dangeard's (2) claim that the vegetative cells of the so-called Protobasidiomycetes are binucleate be confirmed, a very complete parallelism would be demonstrated between the latter and the rusts of the Coleosporium type. Maire says little of the mycelia of the Basidiomycetes which he has studied. The mycelium of *Coprinus radiatus*, however, he says, has uninucleated cells; where the transition from the uninucleated to the binucleated condition occurs he does not say.

Maire describes at some length the division of the nuclei in the basidia. The pairs of nuclei as they divide in the mycelium have each four chromosomes. The fusion nucleus in the basidium shows four chromosomes, both in its first and second divisions. The accuracy of these points can be better judged when the author publishes his figures. He also describes a peculiar and interesting behavior of centrosomes at the time of the formation of the sterigmata. The centrosomes lie at the points on the basidial wall where the sterigmata are to bud out. From these centers fibers differentiated out of the cytoplasm extend to each of the four nuclei which lie at the base of the basidium. Under the influence of these fibers the nuclei gradually approach the vertex of the basidium.

In the light of Maire's observations it becomes probable that the observations of Strasburger (**15**), Rosenvinge (**14**), Istvánffi (**8**), and others, according to which the cells of the Basidiomycetes are regularly multinucleated, were based only on a study of the old vegetative cells of mature sporophores, whose multinucleated condition is secondary and developed in connection with their special enlargement as supporting cells for the variously placed hymenium. Wager says very little as to the nuclei of the vegetative cells and does not describe the condition of the subhymenial cells, though he describes the hyphal nuclei as wandering into the young basidium in pairs (**17**). His descriptions relate mainly to the phenomena in the basidium, but he has figured (17, *pl. XVII, fig. 1*) part of a hyphal cell with a pair of nuclei, which from their position and size suggest that they may be the only nuclei in the entire cell.

An investigation, not yet published, of the structure and development of *Tremella mycetophila*, parasitic on *Collybia dryophila*, by a student in my laboratory, showed that the carpophore of the Collybia contained regularly binucleate cells.

The species of Hypochnus, owing to their simple structure and the loose branching habits of their basidium-bearing hyphae, are very favorable material for the investigation of the nuclear phenomena in the mycelium and young hymenium. Species growing on very rotten wood are specially adapted for good fixation and sectioning. A species agreeing fairly well with *Hypochnus subtilis* Schroet., except that the setae are brown, is abundant in this region. The material in all stages of development from vegetative mycelium to well-developed hymenium with ripe spores can be readily obtained in the fall months.

My material was fixed in Flemming's and in Merkel's solutions, and was sectioned and stained with the safranin-gentian-violet-orange method.

It became apparent at once, as was to be expected, that the cells of Hypochnus, which develops no carpophore of sterile tissue, were regularly binucleate throughout, with the exception of the old stalk cells of the cystids and perhaps the cystids themselves. The conditions in this respect duplicate as nearly as possible those in a rust of the type of *Coleosporium sonchi-arvensis*. As is well known, the genus Hypochnus includes a group of the very simplest known Hymenomycetes. The mycelium ramifies in the substratum of decaying wood or rich earth and produces a simple hymenial layer on its surface made up of basidia alone, or, in some species, with intermingled cystids. As the genus is described, there is nothing to represent a carpophoric structure.

The hymenium is itself quite loose. At first it consists merely of scattered, erect basidium-bearing hyphae, which are readily isolated and show a basipetal habit of branching, very well represented in the figure (*137*, p. 301) of De Bary's *Handbook* (4). The successive development of such erect hyphae and their becoming interwoven and pressed together results in the formation of the hymenial layer. At first these erect fertile hyphae can be readily isolated and studied in the living condition, as was done by the older authors, the entire system of branches from a single main trunk being thus very easily and accurately worked out, as shown so well in the figure referred to above. In stained sections, too, these earlier tree-like fertile branches stand out with beautiful distinctness, and show basidia in all stages of development, and with their nuclei in all conditions. The order of succession in the development of the basidia can be traced with perfect accuracy. Later, as the hymenial layer becomes more dense, these individual twig systems can be no longer differentiated, though there is no question that the further development consists in nothing more than the pushing up of additional erect hyphal branches among those earlier developed. The species investigated forms a delicate frost-like growth on the surface of very well rotted logs, and should be collected in damp weather. The substratum was so soft as to offer no special resistance in cutting, and it was therefore possible to take off thin slices of the substratum several millimeters square and fix them with practically no disturbance of the fungus.

The mycelium was found ramifying through the decayed wood cells in all directions. The peculiar clamp connections, described by Hoffman (7) between adjacent cells of a hypha, were abundant. In fact, they seemed to be present at one or both ends of nearly every cell. Brefeld (1) has described these clamps in Coprinus as originating in a tube which pushes out from the end of one cell, bends over, and fuses with the end of the next adjacent cell. Brefeld finds that before the clamp tube fuses with the second cell it is separated from its parent cell by a septum. Later he finds also that a second septum is put in, replacing the walls broken down in the fusion. The mature clamp is thus cut off by a septum from each of the two cells which it connects. I have not observed the development of these clamp connections in Hypochnus, but in the mature condition as they are universally found in my material they show but one septum, and that apparently on the cell from which the clamp-tube originated. The significance of these structures is not clear. Presumably they facilitate the transfer of excess food materials and moisture from one cell to another, but just how they are adapted to this function is not evident from their structure. I have not observed in Hypochnus the granules or plates on the septa between adjacent cells which are so common in many Basidiomycetes and Ascomycetes (**5, 7**), and which probably indicate the location of some sort of pores for communication between the hyphal cells. In Coprinus, as we shall see later, such plates are present in great abundance, and have a very characteristic appearance.

The mycelial cells of Hypochnus vary considerably in length, though of fairly constant diameter in the mature mycelium. A cell of about average proportions is shown in *fig. 1*. In the specimens studied the hyphae formed no dense wefts or strands in the substratum. On the whole it seemed rather loose and sparse. The vessels of the decaying wood with their large empty cavities afforded a favorable opportunity for studying the hyphal cells, the hyphae cutting through these cavities in all directions, but never filling them densely. The mycelial cells are practically without exception binucleate. The two nuclei generally lie rather close together and near the center of the cell, but they may be at opposite ends, or otherwise placed in the cell. *Fig. 10* shows that, however the pair may be located in the cell, they are generally rather close together. These nuclei show, in well-stained preparations, a nucleole and chromatin sharply differentiated, and are sharply bounded by a nuclear membrane.

Fusions (**6**), such as have been described by many observers between cells that are near together or in contact, are quite common in Hypochnus. There is no clear evidence that in this case they have any other significance than as channels for the interchange of food materials, etc. Such a fusion is shown in *fig. 10*, in the lower right-hand part of the subhymenial tissue.

The mycelium of Hypochnus in the case studied forms no felt of hyphae on the surface of the substratum, apparently, until the time has arrived for the formation of basidia. That is, through its purely vegetative existence it is entirely buried. When the hymenium is to be formed, erect aerial branches are pushed up, and branch into the tufts referred to above as figured by De Bary. The main axes of these tufts are never close packed, but arise from the substratum at relatively considerable distances from each other. Their abundant interlacing branches, however, form a dense hymenial layer higher up. We have thus a condition in this respect resembling a diminutive forest, or growth of shrubbery, whose branches and twigs are densely interlaced, while the trunks from which they arise are separated by relatively wide open intervals. This is shown, with a modification noted below, in the semi-diagrammatic *fig. 10*. The whole mass of the hymenium here shown is supported by the three hyphae arising from the substratum. The figure, to be sure, is of a section, so that part of the hymenial cells belong really to other twig systems arising above or below the level of the section. A corresponding portion of the branches of the two main hyphae figured lie also above or below the section, and hence do not appear, so that the proportion of peripheral branches to main axes as shown is not materially affected. The case is further complicated frequently by proliferation from the surface of an old and apparently exhausted hymenial layer. This is the condition shown in the figure in question, where the hymenium which shows the immature basidia has been superimposed on the surface of an older one. The upper surface of the latter is very clearly indicated, and it is at once seen that the same method has been employed in forming the second hymenial layer as in the first case. The old hymenium has become the substratum out of which arise scattering vertical hyphae, which by their copious branching then form the new hymenium. The old hymenium is seen to consist of a mass of hyphal cells very poor in content which have replaced the basidia. The latter, as in all true Basidiomycetes, collapse soon after their spores are ripened.

Whether this habit of forming new hymenia by proliferation from those already exhausted is due to alternation of favorable and unfavorable conditions of moisture, etc., I am not sure. It is also uncertain whether a single hymenial layer may go on indefinitely producing new basidia to replace those which have ripened their spores as long as conditions are favorable for growth. Such continuous unlimited growth is quite possible by the basipetal method of branching shown in De Bary's figure. It would not result, however, in the formation of a second new hymenium, such as is shown in *fig. 10*, and it seems quite probable that the latter has been secondarily formed after a check to growth due to cold or drouth sufficient to stop the formation of basidia in the first hymenium. Whether a third hymenial layer might be developed in the same manner I have not determined. No such case has been found in the material so far studied.

It is interesting to note that, although the structures described for Hypochnus are extremely delicate and of microscopic size, the process of forming the new hymenial layers is essentially the same as that found in the larger stratified Polyporeae. Hypochnus, as we see, is really stratified, though not so described, and though lacking any markedly differentiated pileus. In the large woody fungi of the stratified Fomes type the new hymenial layers are formed as layers of pores and in successive seasons. But the method of their development by proliferation of new basidium-bearing hyphae from the old mycelial tissue of the previous season is essentially similar to that described above (so far as is known), except that the process is on a larger scale in Fomes, and the layers formed are of visible thickness.

If we look more closely now at the actual method of branching of the basidium-bearing hyphae of Hypochnus, we find, as indicated above, that the new branches are regularly produced basipetally. Each end cell becomes a basidium, and before this apical basidium is more than half developed the cell beneath it pushes out a new basidium (*fig. 4*) which grows up beside the first. Later a second (*fig. 3*), and in some cases even a third, basidium may bud out on different sides and just beneath the basal wall of the first basidium. This gives the appearance of a stalk cell bearing a cluster of basidia at its apex. Since these basidia are pushed out successively, however, and their formation consists essentially in a process of cell division, the whole cluster represents really a basipetal series. The stalk cell becomes successively, at least as far as the nuclei are concerned, the sister-cell of each developing basidium as it is cut off. At the same time, from cells below the stalk cell of the first basidium lateral branches may be given off and grow to a length of from one to several cells, according to the level at which they arise from the main axis. Still further branchlets may arise from the cells of these lateral branches. In the end the apical cells of all these branches become basidia, and just beneath the septa which cut off these basidia one or two further basidia bud out. The basidium terminating the main axis of such a system is always the oldest and ripens first. The clusters produced on the lateral branches push up to the height of the main axis, but not higher, and ripen successively. The whole process is easily understood from a study of De Bary's figure and *figs. 2–4, 7, 9, 10*. With reference to their method of branching, we may compare such hyphal systems with their basidia to a flat topped cyme among the flowering plants.

The entire fruit body of Hypochnus is composed thus of a series of such cymosely branched hyphal systems, whose branches are intricately interlaced and packed together. Such a hymenium is strictly a compound structure made up of a series of similarly branching fertile hyphae. The only unity to be found in it lies in the fact of its possible origin from a single mycelium produced by the germination of a single basidiospore. As a matter of fact, it is highly improbable that such unity as this ever really exists in any given case. It is much more likely in the majority of cases that mycelia from different spores are mingled in the substratum, and that any particular hymenial layer is made up of fertile branches from different mycelia. Their ability to combine into the even homogeneous films extending over many square centimeters would be wholly due to their similar environment and their mutual interaction, and not to any unity of origin, since each mycelium must be regarded as an independent plant.

It is plain that this conception of the Hypochnus hymenium as a compound having no strict individuality is of great importance for determining the morphological value of the more complex carpophores of the higher Basidiomycetes, and in comparing them with the fruit bodies of the Ascomycetes, which arise typically at least as units from the cell or cells of a sexual apparatus, so far as their fertile ascus-bearing hyphae are concerned.

The cells of the hyphae which occupy the place of the old hymenium in *fig. 10* are very poor in protoplasmic content. Among them are the empty and partly collapsed cell walls of the old basidia, which are scarcely recognizable, owing to the growth and crowding of the new mycelial tissue. How this new growth arises from the subbasidial tissue I have not been able to make out. The old basidia and their connections cannot be traced at all definitely at such a stage as is represented in *fig. 10*. There is no reason for anticipating anything especially noteworthy in the details of such a proliferation. The fact that it occurs, however, is a still further point of difference between the Ascomycetes and the Basidiomycetes. The former never show such secondary hymenia developed on the surface of old hymenia. The ascogenous hyphae show frequently a protracted, but none the less ultimately a definitely limited, growth, resulting in a characteristic fruit body, the ascocarp; while the merely vegetative hyphae which bear the basidia may prolong and repeat at intervals the development of new hymenial layers.

The stromata of Diaporthe, and other similar types which may show more or less secondary growth, are not ascocarps, but vegetative mycelia bearing ascocarps imbedded in their substance (18).

The cells of the old hymenium, like those of the mycelium buried in the substratum, contain regularly two nuclei. These are usually placed rather close together, as noted above. The apical cells which are to form basidia contain regularly two, and only two, nuclei. I have found no exceptions to this rule, though doubtless such occur as abnormalities, as pointed out by Maire. The young basidium is also regularly separated from its stalk cell at an early stage, so that in Hypochnus there is no chance for such a migration of pairs of nuclei as Wager thinks probable. The conditions are especially favorable in Hypochnus for determining this point. In the subhymenial tissue of the agarics the hyphae are so densely packed, in many cases forming a pseudo-parenchyma, that it is difficult to trace the path of a single hypha for any distance, or to determine just what the connections of the basidium at its base may be. In Hypochnus the loose branching makes it possible to determine just how and when each basidium has been formed and cut off, and it is certain for this case that Maire's general statement that two, and only two, nuclei are cut off in the young basidium is true, as opposed to those of Rosen (**13**) and Wager (**16**). The stalk cells also contain two nuclei. The conditions just described are well illustrated in *figs. 2, 3*, and *7*.

Whether the two nuclei of the cell from which the basidium is cut off divide simultaneously, and whether simultaneous nuclear division ("conjugate nuclear division" of Poirault and Raciborski [**12**]) occurs generally in the formation of new cells in the mycelium, I have been unable to determine with certainty. That this is the rule in the case of the binucleated cells of the rusts there can be no doubt. The work of Dangeard and Sappin-Trouffy (**3**) and Poirault and Raciborski (**12**) establishes this fact and I have been able to confirm for myself their results on this point. As to the details of the so-called conjugate division, however, the figures so far must be considered as very unsatisfactory. The evidence presented by Sappin-Trouffy and by Poirault and Raciborski is not at all convincing on any points further than that the nuclei lie side by side and divide simultaneously.

In the same way I have also found great difficulty in obtaining satisfactory figures of the nuclear division in the cell from which the basidium is cut off, and prefer to reserve judgment on the whole process until more satisfactory methods of preparation have been worked out for this material. Maire in both his papers referred to describes the nuclear division as of the conjugate type, the same as in the rusts. Still the evidence is not clear, and in the formation of the secondary basidia, which bud out from the stalk cell of the one first cut off, figures are not uncommonly found which point to a different process. For example, in *fig. 4* it is seen that one nucleus is already in the young basidium, while one has remained in the stalk cell. It would seem as if the next step would be the division of these nuclei *in situ*, giving two sister-nuclei, for the young basidium and another pair of sister-nuclei for the stalk cell.

The stalk cells contain quite regularly two nuclei after the basidia have been cut off (*figs. 2* and *7*). I have also found a number of cases in which the two nuclei of the mother-cell of the first basidium of a cluster were dividing simultaneously. The figures, however, were dense and unsatisfactory, resembling the figures of the conjugate division in the rusts referred to above; and, while affording evidence of simultaneous division, are not, in my opinion, to be regarded as at all typical. Maire states that all the Hymenomycetes studied by him show four chromosomes in these mycelial nuclei, but in the absence of figures it is difficult to judge of the evidence on which this conclusion is based.

The pair of nuclei in a young basidium grow rapidly with the growth of the basidium. This very notable increase in their size seems to have been regarded by Wager as suggesting the likelihood of repeated nuclear fusions, but the phenomenon is not different from that in Synchitrium, where with the growth of the fungus cell the nucleus grows until from being one of the minutest of nuclei it becomes much larger than the nuclei of the host-plant cells which surround the fungus. The significance of such changes in nuclear volume is not clear, but they are regularly correlated with increased size in the cell to which the nucleus belongs.

Fusion takes place generally when the basidium has reached from one-half to two-thirds of its mature size, though wide variations from this rule may be observed. The process can be observed readily in all stages. The nuclei become flattened upon each other. The nuclear membranes disappear over the area of contact and the nuclear cavities become continuous. The fusion nucleus shows a constriction for some time around the area of union. This furrow gradually disappears and the nucleus is evenly oblong oval in outline. Later it becomes more spherical, regaining the shape which it had before fusion took place.

Just how the chromatin masses are combined cannot be made out. They become apparently continuous in the region of contact as soon as the membranes have disappeared. Later no line of demarcation between the two can be made out. Still I am inclined to believe that the appearances of homogeneity are not to be taken as conclusive evidence on this point. There seems little doubt that the nucleoles fuse. They are conspicuous, sharply defined objects clearly differentiated as clear red spheres in the blue chromatin throughout the whole process of fusion. Each of the nuclei before fusion appears with astonishing regularity as possessed of one nucleolus (*figs. 2–4*), and just as regularly the spherical fusion nucleus shows a single and frequently much larger nucleolus. During the earlier stages of fusion the two nucleoli are still present (*figs. 5, 6*). They are frequently found later close together and pressed upon each other, though such figures as these are not to be considered as having great weight in view of the irregularity in shape which nucleoli frequently show. The fusion nucleus increases still further with the further growth of the basidium. It lies generally in the mature basidium at about two-thirds the distance from the base to the apex.

The stages in the division of the nucleus have been very fully described by Wager. They are abundant and easily found in Hypochnus. In general, the karyokinetic figures agree with those found in the division of the ascus nuclei. A more detailed account of the stages I shall give later in another connection. The fusion nucleus regularly migrates to the apex of the basidium before it divides. The spindle is always transverse to the long axis of the basidium. In the equatorial plate stage and a little later the chromosomes are well individualized on the spindle, and the number seems to be at least from eight to twelve before the anaphases begin, as shown in *fig. 8*. Maire gives four as the number of chromosomes appearing in the division of the nucleus of the basidium in the long series of forms studied by him. It is certain that there are many more than four chromosomes in the nucleus of the basidium of Hypochnus. The second division immediately follows the first, and the spindles here are also transverse to the long axis of the ascus.

The four daughter nuclei then retire downward to about the middle of the basidium, while the basidium stretches up still farther in length, and the sterigmata bud out around its apex (*fig. 9*).

Maire has described strands of fibers (kinoplasm) connecting each nucleus with the point of origin of a sterigma. He interprets this as signifying the presence of centrosomes on the walls of the basidia at the point at which the sterigmata are to form, and considers that the fibers are concerned in drawing the nuclei up into the spores which bud out on the tops of the former. I have observed in some cases in Hypochnus such fibers extending from the nuclei up into the developing sterigmata, but their origin I have not been able to determine. The fully formed spores contain a single nucleus and are oblong, and, as a rule, slightly flattened on one side.

I have also investigated the nuclear phenomena in a species of the Agarics, *Coprinus ephemerus*. Sections through the young fruit body reveal at once the conditions described by Maire. The stipe and pileus consist of large multinucleate cells with extremely numerous nuclei. A cross-section of a small group of cells from the stipe is shown in *fig. 12*. The cells in cross-section are seen to be almost circular, and of extremely variable size. The entire cross-section of the stipe shows it to be made up of large cells, with very much smaller cells intermingled with them. The center of the stipe in these species is hollow, and the hollow space is bounded by a layer of these smaller cells. A longitudinal section of the stipe shows that the cells shown in *fig. 12* are immensely elongated and quite regularly parallel, and in turn are combined end to end to form parallel hyphae.

These sections show also that the cells contain very numerous nuclei, the number varying with their size. In very young carpophores the cells are quite rich in protoplasmic content, the protoplasm being largely aggregated in a central column running the length of the cell, in which most of the nuclei lie. As the cells elongate with the growth of the carpophore, they become proportionally poorer in content, and the nuclei are distributed more evenly.

The tissue of the pileus is also seen to be made up of large multinucleate cells. *Fig. 13* represents a group of cells from the peripheral region of the pileus, and shows four cells prolonged into blunt, spine-like processes, which project outward from the upper surface of the cap. The whole surface of the cap is covered with similar spines in this species. Between these spine-cells are the columnar outer cells of the pileus, forming a sort of epidermis, and beneath these again are mingled large and small cells. All of these different types of cells are multinucleate, but are not as large and do not contain as numerous nuclei as the stipe cells. They are also relatively poor in protoplasm, and contain a single large central vacuole, or numerous smaller ones.

It is doubtless the study of these cells of the vegetative part of the carpophore that has led to the conclusion of Strasburger, Wager, and others, that the vegetative bodies of the Basidiomycetes contain typically many nuclei. If we study the cells which are forming the gills of a carpophore of Coprinus, however, an entirely different condition of things is found. The gills originate as plates on the inner surface of the pileus as soon as it is differentiated around the upper end of the stipe. The separation of the pileus from the stipe is, indeed, brought about, in part at least, by the development of these plates. The first formed gills are attached along their entire margin, for a time at least, to the surface of the stipe, and the cavities lined by the hymenial surfaces originate as thin, vertical, intercellular spaces, placed radially about the stipe, as is very well shown by transverse sections through the upper end of the young carpophore. These earliest formed gills later split off at their inner edges from the stipe as the pileus expands and gradually assumes the umbrella shape. The intercellular spaces, which are the interlamellar spaces, become wider with the growth of the pileus, and later formed gills arise in these spaces as further outgrowths from the under surface of the pileus. The study of the nuclei in these later formed gills is especially easy. The mature gill consists of two hymenial layers, with a vegetative supporting layer between them, the trama. In the young gill these parts are not sharply distinguished. It is formed simply as a ridge made up of hyphal branches, which grow radially inward and slightly downward. I am inclined to think that these hyphae branch just as do the hyphae in the hymenium of Hypochnus, in something like a cymose fashion, the apical cells being continually pushed to the side by branches produced from the cell beneath. The growth of the hyphae progresses radially inward and downward until the width of the mature gill is reached. The branches which are pushed out right and left terminate their growth almost immediately by the formation of basidia. These basidia intermingled with sterile cells, which are apparently their morphological equivalents, together make up the hymenial layers. In the young gill the hyphae are packed densely together throughout. As they grow older the central cells become separated by intercellular spaces in many cases, and thus form the looser tissue of the trama.

Since the axis of growth of the gill is radially inward and also downward, sections in an exactly transverse plane through the young carpophore do not show the method of growth as well as those which are slightly oblique. Such sections, inclined a few degrees to the long axis of the carpophore, lie in the axis of growth of the hyphae of the gills and give the largest percentage of cells cut longitudinally in the young gill. This is of course true for the young gills on one side of the carpophore only, those on the opposite side, as will be readily understood, showing the hyphal cells cut more nearly transversely than in exactly transverse sections of the entire carpophore. *Fig. 11* shows the section of a very young gill taken from such an oblique section of a carpophore. The cells are nearly all cut longitudinally, and the method of their growth and branching is fairly well indicated. Still even here certain hyphae are seen to have turned up or down and out of the plane of the section, so that the axial hyphae of the base of the gill and the end cells are not connected to form a continuous hypha in the section.

The conspicuous fact here is that all the cells of the gill are binucleated. Following backward through the base of the gill, we should pass suddenly into tissue of the pileus exactly similar to that shown in the inner portion of *fig. 13*. It is not easy to trace a single hypha from the one tissue to the other; still there seems no doubt that the binucleated cells are the end ramifications of hyphae which farther back in the pileus and stipe are composed of multinucleated cells. In other words, as Maire's work shows, the ends of the hyphae which are to reproduce the organism by means of basidia and basidiospores have maintained the binucleated condition, while the mass of the hyphal cells, having only the functions of support and transportation of water, etc., to the reproductive cells to provide for, have become enlarged and multinucleated as a result of nuclear divisions unaccompanied by cell division. The reproductive series of cells, the *Keimbahn* of various authors, is thus sharply distinguished from the purely vegetative and somatic series by the number of nuclei which they contain respectively. In very early stages in the development of the carpophore the differentiation of the two types of cells is present and continues through its whole development. Whether the multinucleated cells increase in number by cell division, as do the binucleated, is not easy to determine. It seems highly probable, however, that, like the internodal cells of Chara and Tradescantia, they may have lost the capacity to divide, the increase in number being entirely provided for by the division of the binucleate cells. The development of the carpophore, as pointed out by De Bary (4, p. 49) and others, is no doubt to be interpreted as consisting in the growth of hyphae, and it seems highly probable that the hymenium represents the end cells of the hyphal branches which are to be traced back through the pileus into the stipe, and so connected with the mycelial branch or branches from which the carpophore sprung. The growth would then be largely apical for these hyphae, the older cells lying in the pileus and stipe, and the youngest, latest formed, in the hymenium. That all the hyphae of the stipe have end cells in the hymenium is, of course, not necessarily assumed, though it is not improbable, in view of the wide expanse of the latter, that this is the case.

What the condition of the mycelial cells is in the species of Coprinus studied I have not been able to determine. Maire says that the mycelium of *Coprinus radiatus* is made up of uninucleated cells. The base of the stipe of *C. ephemerus* is surrounded by a mass of hyphae with multinucleated cells; whether these, however, represent the condition of the primitive mycelium from which the carpophore sprung I am by no means sure. It may well be that in the work of collecting and transporting material for the young carpophore the mycelial cells may have become enlarged and secondarily modified as to the number of their nuclei. There seems little doubt, however, that the condition in Hypochnus represents a very primitive condition for the Basidiomycetes, and that, as Maire maintains, all multinucleated cells in their vegetative bodies have been secondarily produced. How the condition of things in *C. radiatus* with its uninucleated mycelial cells is to be connected with that in Hypochnus is not plain. It is quite possible, of course, that the uninucleated condition represented in the basidiospore may be perpetuated for longer or shorter periods of development in different cases.

The subhymenial cells of *C. ephemerus* show very conspicuous disc- or saucer-shaped bodies arranged in pairs on opposite sides of their walls. These bodies stain deeply and are very numerous, lying on all sides of the hyphal cells wherever the walls of adjacent cells are in contact. *Fig. 14* shows two pairs of such bodies and their relative position with reference to the cell walls. Strasburger (**15**, p. 335) has described similar structures. They doubtless mark points where the walls are perforated in some fashion, thus establishing connection between adjacent cells. I have described such bodies for Pyronema (**5**), and also spherical granules which may have a similar significance.

Brefeld (**1**) has described the carpophore of *C. stercorarius* as arising from a single hypha of the vegetative mycelium. This hypha produces a richly developed system of branches which arrange themselves ultimately into the parts of the young carpophore. Vegetative growth of the mycelium and formation of the carpophore are thus seen in this case to be rather sharply separated, and it might well be that at this point the transition from uninucleated to binucleated cells is effected. However, no such differentiation of mycelial growth and carpophore formation exists in Hypochnus, whose mycelium is binucleated throughout in its mature condition. Maire says nothing as to the stage when the binucleated cells first appear in Coprinus. If his statement is true, that the mycelium of the latter plant is made up of uninucleated cells, it differs, as noted, from Hypochnus in this respect. Whether Hypochnus or Coprinus would represent the more primitive condition in this particular is not easily to be determined with certainty. Most mycologists, however, have agreed so far in regarding the Tomentelleae as primitive Basidiomycetes, and the Agarics as among the most specialized forms; and, in the absence of positive evidence to the contrary, this seems the most natural assumption. Still, it is quite possible that the Hypochnus type may be degenerate in certain particulars and may have lost a mycelium of uninucleated cells, such as Maire reports for *Coprinus radiatus*. Just where in this case the binucleated condition would arise is not clear. There is little doubt, however, that the development of multinucleated cells in the group is correlated with the origin of large and complex carpophores, and that in lacking such cells Hypochnus represents a more primitive type.

Basing his conclusion on the resemblance of certain conidiophores of the Ascomycetes to the so-called protobasidia, Massee (11) seeks to connect the Basidiomycetes with the Ascomycetes. He believes that it is in asexual stages of the latter that the ancestors of the Basidiomycetes are to be found, accepting Brefeld's conclusion that the basidium is a conidiophore which has become determinate in the number of spores which it produces. Massee points out that the conidiophores of these asexual forms of the Ascomycetes conform very closely to the type of the Auricularia basidium. From the fact that about thirty species of the genus Stilbum and several species of the genus Tubercularia are known to be the asexual reproductive forms of species of the ascus-bearing genera Nectria and Sphaerostilbe, while other species of the former genera have never been found connected with ascus-bearing forms, Massee argues that these latter asexual forms are probably becoming independent and have lost the ascus-bearing stage permanently. The conidiophores of the Stilbum form of *Sphaerostilbe microspora* Cooke and Massee are already typical protobasidia. The same is true of *Tubercularia volutella*, whose so-called conidiophores are likewise protobasidia. The examination of a large number of forms of Stilbum and Tubercularia shows that a series of gradations from forms with typical protobasidia, *i. e.*, two or three short swollen fertile cells, to forms with unmodified conidiophores, *i. e.*, two or three long narrow fertile cells forming the end of the conidiophore, can be made out. As a rule, the conidiophores are found on forms which are still associated with an ascus-bearing stage. *Sphaerostilbe microspora*, however, is an exception to this rule. Isaria, many of whose forms are connected with Cordyceps, while others are, the author believes, independent species, shows also typical protobasidia in *I. pulcherrima* Berk and Broome; while other species show the transition from protobasidium to conidiophore. Massee believes that Möller's protobasidiomycete *Pilacrella delectans* stands close to the Isaria forms. Massee concludes that the "protobasidiomycetes as a group are derived from the conidial phase in the life-cycle of ascigerous fungi; the evolution is effected by the disappearance of the ascigerous form of reproduction, whereby the conidial phase assumes the standard of a species; this change being contemporaneous with the gradual conversion of the so-called conidiophore to the typical basidium or spore-bearing organ." He does not accept Juel's proposition to transfer such forms as *Stilbum vulgare* Tode into the Protobasidiomycetes, holding that the transition stages are so characteristic that no hard and fast line can be drawn between the forms with protobasidia and those with conidiophores.

Massee follows Möller in denying that the true basidium with apical spores has been derived from the septate basidium. He believes that these non-septate basidia have been developed from conidia of the type seen in Matruchotia, Botrytis, Polyactis, and others, through the non-septate basidium with lateral spores of the type seen in Tulostoma. Brefeld's Auto- and Protobasidiomycetes are both derived from the Ascomycetes, but as separate and distinct offshoots. Whether each of the two groups is to be further regarded as polyphyletic and consisting of separate series derived respectively from the forms Stilbum, Isaria, etc., the author does not state.

In the light of the facts described by Maire and myself, as recorded above, all such conclusions as these of Massee are seen to be extremely uncertain. The typical binucleated condition of mycelial cells is as yet unknown in the Ascomycetes, and until a transition from the coenocytic to the binucleated condition has been discovered in the forms discussed by Massee, his argument can be regarded as of little value. The widespread occurrence of regularly binucleated cells in the Basidiomycetes, with the additional evidence that these cells reproduce by conjugate division and constitute the reproductive series (*Keimbahn*) in each individual through at least a considerable part of its life-history, leading up to the formation of basidia, while no such binucleated cells are found in the Ascomycetes either in vegetative or ascogenous hyphae, shows that the two groups are widely separated phylogenetically. In the face of such differences, resemblance of outer form and method of spore-formation between conidiophores and basidia must be regarded as superficial and of uncertain value, and as wholly inadequate evidence for the conclusion Massee wishes to draw. On the other hand, it is quite clear that the binucleated condition in the hyphae of both groups still further strengthens the evidence for the relationship between the rusts and Basidiomycetes. The nuclear fusions occurring in the teleutospore and basidium are seen also to be directly comparable processes, and there is a strong presumption that in the Basidiomycetes, as in the Uredineae, this fusion is not between sister nuclei, but nuclei which by the process of conjugate division have remained distinct through long periods of vegetative growth. Maire affirms unequivocally the existence of conjugate division in all the forms he has studied.

It must not be assumed without further evidence that the rusts are primitive Basidiomycetes. Their parasitic habit is against this view, and the evidence from the resemblance of a sorus of Coleosporium to a true hymenium cannot be considered as very conclusive. Still, the binucleate condition of the hyphal cells suggests very strongly that rusts and Basidiomycetes must have arisen from some ancestral type characterized, at least in some stage of its development, by the possession of binucleate instead of uninucleated or multinucleated vegetative cells. How this binucleated condition arose originally is not at all clear. The view of Raciborski that the binucleated cells of the rust represent a prolonged vegetative stage interposed between two phases of a sexual act, namely the cell fusion and the nuclear fusion, is suggestive as a hypothesis, but it is nothing more.

The question as to the nature of the spermogonia and their relation to the aecidia of the rusts remains still unsettled, and

De Bary's view as to the relationship of the rusts and Ascomycetes must be given up.

Further, it is plain, in view of the above facts, that the processes of nuclear fusion in asci and basidia are, so far as the present evidence goes, entirely unrelated morphologically, and that ascus and basidium are not to be brought into any common category as oogonia, as is attempted by Dangeard. Neither the cells of the ascogenous hyphae nor the vegetative mycelial cells of the Ascomycetes are binucleated in any case so far investigated, and there is no suggestion of any close morphological resemblance between ascus and basidium. The significance of the fusion is not entirely clear in either case, but no light is thrown on this point by endeavoring to bring together such heterogeneous structures.

It is highly probable that the nuclei of the basidium, as of the teleutospore, are of widely separated ancestry. It is clear in the case of Pyronema, at least, that the nuclei which fuse in the ascus are not sister nuclei. Further than this the resemblance in this respect does not extend. The origin of the ascocarp in a sexual process is analogous to nothing which has so far been discovered in the Basidiomycetes. It may well be that both the origin and the physiological significance of the fusions in ascus and basidium are entirely distinct.

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EXPLANATION OF PLATE I.

All figures were drawn with the aid of the camera lucida, *figs. 1–4, 7,* and *9* with the Zeiss apoch. objective ap. 140, oc. 8, and *5, 6, 8,* and *14* with oc. 18.

Hypochnus subtilis.

FIG. 1. Hyphal cell showing two nuclei and the characteristic clamp connections.

FIG. 2. Young basidium and stalk cell, each with two nuclei.

FIG. 3. Basidium with two younger basidia budding out beneath it.

FIG. 4. Young basidium with two nuclei; stalk cell with young basidium budding out and containing a single nucleus.

FIG. 5. Young basidium with its two nuclei fusing.

FIG. 6. Later stage in the fusion of the nuclei of the basidium.

FIG. 7. A pair of basidia in one of which nuclear fusion has taken place, while in the other it has not yet begun.

FIG. 8. First division of the nucleus of the basidium.

FIG. 9. Two basidia, one with four spore nuclei and sterigmata, and one with two nuclei before fusion.

FIG. 10. Semi-diagrammatic section of the aerial portion: the upper layer is a young hymenium with basidia in various stages of development; to the right is a basidium with nearly ripe spores, each with a single nucleus; next to this basidium on the left is a young, only partly grown cystid, with very densely stained content; below the hymenium is a layer of vegetative tissue which has replaced a former hymenium; this is borne on three hyphal branches springing from the substratum below. × 800.

Coprinus ephemerus.

FIG. 11. Section of young gill made up of binucleate cells. × 800.

FIG. 12. Transverse section of a group of cells from near the upper end of the stipe of a very young fruit body. × 1000.

FIG. 13. Section of a bit of the peripheral tissue of the pileus, showing the columnar outer layer and four of the elongated papilla-cells.

FIG. 14. Parts of the walls of three sub-hymenial cells showing the pairs of bodies marking the position of intercellular pores.